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Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein G_S

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McCune-Albright syndrome (MCAS) is a sporadic disease classically including polyostotic fibrous dysplasia, café au lait spots, sexual precocity, and other hyperfunctional endocrinopathies. An activating missense mutation in the gene for the alpha subunit of G_S, the G protein that stimulates cyclic adenosine monophosphate formation, has been reported to be present in these patients. The mutation is found in variable abundance in different affected endocrine and nonendocrine tissues, consistent with the mosaic distribution of abnormal cells generated by a somatic cell mutation early in embryogenesis. We describe three patients with MCAS who had profound endocrine and nonendocrine disease and who died in childhood. Two of the patients were severely ill neonates whose complex symptoms did not immediately suggest MCAS. A mutation of residue Arg^{201} of $G_{s\alpha}$ was found in affected tissues from all three children. A review of the literature and unpublished case histories emphasizes the existence of other patients with severe and unusual clinical manifestations. We conclude that the manifestations of MCAS are more extensive than is generally appreciated, and may include hepatobiliary disease, cardiac disease, other nonendocrine abnormalities, and sudden or premature death. (J PEDIATR 1993;123:509-18)

McCune-Albright syndrome is a sporadic disease classically defined by polyostotic fibrous dysplasia, café au lait spots, sexual precocity, and other hyperfunctional endocrinopathies.[1-4] Endocrine tissues that function autonomously in MCAS include the gonads, thyroid, adrenal cortex, and pituitary somatotrophs. The sporadic occurrence of MCAS, its variable presentation, and the curious pattern of skin pigmentation led to the hypothesis by Happle[5] that this disorder is secondary to a dominant somatic mutation occurring early in embryogenesis. According to this model, patients with MCAS have mosaicism for the mutant gene, and the particular constellation of abnormal findings in each patient is dependent on the particular distribution of mutation-bearing cells.

Cyclic adenosine monophosphate is known to stimulate

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ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AST	Aspartate aminotransferase
GGT	γ-Glutamyltransferase
G_S	Stimulatory guanine nucleotide binding protein
MCAS	McCune-Albright syndrome

the growth or function of tissues affected in MCAS.[6-9] A mutation in the gene for the alpha subunit of the stimulatory guanine nucleotide binding protein G_S, the G protein that stimulates cyclic AMP formation, has been reported in five patients.[10,11] The mutation was found in variable abundance in several affected tissues, consistent with the mosaic model. The single-base mutations that were found encoded the replacement of the arginine 201 residue of $G_S\alpha$ with either cysteine or histidine. The G_S protein acts as a molecular switch and timer, relaying information from hormone-bound receptors to the enzyme adenylate cyclase when it is in its active, guanosine triphosphate–bound state.[12] Deactivation of the G protein normally occurs when the γ-phosphate of guanosine triphosphate is hydrolyzed by an intrinsic guanosine triphosphatase. Substitution of the arginine residue with a different amino acid inhibits this guanosine triphosphatase activity and leads to prolonged stimulation of adenylate cyclase and production of cyclic AMP in the presence of little or no hormone.

Nonendocrine abnormalities and early death were described in several of the first reported cases of MCAS and in sporadic reports thereafter, but the significance of these accumulated observations has rarely been addressed.[4,13-16] The syndrome is generally considered nonfatal, and two long-term follow-up studies did not suggest that patients were at increased risk for nonendocrine disease or early death.[17-19] Three of four patients in our first report had significant nonendocrine abnormalities, including chronic liver disease of unknown cause, thymic hyperplasia, gastrointestinal polyps, and cardiopulmonary disease.[10] Two patients had unexplained sudden death. Mutations were present in specimens of affected nonendocrine tissue, including liver and heart.[10]

We now describe three additional patients with MCAS who had profound endocrine and nonendocrine disease and who died in childhood. A mutation of residue Arg^{201} of $G_S\alpha$ was found in affected tissues from all three children. A review of the literature and unpublished case histories highlights the existence of a severely affected subset of patients with MCAS who may be at risk for nonendocrine disease and sudden or premature death.

METHODS

Relevant medical records, surgical pathology summaries, and autopsy reports were reviewed for each patient. DNA was isolated from paraffin-embedded tissue specimens obtained from each patient.[10] Slides stained with hematoxylin and eosin were reviewed and used to identify regions of interest. Fibroblasts from patient 2 were grown from a nonpigmented skin specimen obtained at the time of surgery. A short fragment of the $G_S\alpha$ gene (exon 8) containing the Arg^{201} codon was amplified with the polymerase chain reaction, and mutations were identified with allele-specific oligonucleotide hybridization as previously described.[10] This technique measures the specific binding of short, radioactively labeled oligonucleotide probes that either match the wild-type DNA sequence exactly (R201) or contain a single-base substitution (R201H or R201C). Under stringent washing conditions, only the probes that exactly complement the immobilized DNA will remain bound to the filter. DNA isolated from paraffin-embedded normal tissue was used as a negative control, and DNA from pituitary tumors with known Arg^{201} mutations was used as a positive control.

RESULTS

Patient 1. Patient 1, a white male infant, was delivered by cesarean section at 36 weeks of gestation because of fetal tachycardia. Apgar scores were 8 and 9 at 1 and 5 minutes. Birth weight, length, and head circumference were at the 50th percentile. A large, faint café au lait spot was present over the sacrum. At 2 days of age the patient was transferred to a neonatal intensive care unit because of unexplained tachycardia, heart murmur, jaundice, elevation of serum liver enzyme values, and fever.

A chest radiograph showed cardiomegaly, a multiple gated acquisition scan showed a diminished ejection fraction of 55%, and cardiac catheterization revealed mild right ventricular hypertrophy. Fever and tachycardia persisted, but culture results were negative. At 4 weeks of age the patient was noted to have hypertension (systolic pressures 100 to 125 mm Hg, diastolic pressures 85 to 105 mm Hg), and hypokalemia (potassium value, 2.4 mmol/L). Plasma renin activity, aldosterone, and urinary potassium excretion were elevated. Primary renal disease was suspected, but renal imaging studies revealed only duplication of the right ureter. Multiple medications were required to control hypertension.

An endocrine consultation was obtained at age 3 months. The thyroxine value was 225 nmol/L (17.5 μg/dl; index 21), the triiodothyronine value was 3.0 nmol/L (194 μg/dl; index 211), thyrotropin was suppressed and not stimulated by thyrotropin releasing hormone, iodine-123 uptake was normal, and thyroid stimulating immunoglobulins were undetectable. Abnormal skeletal radiographs and gallium scan at 2 months had initially been interpreted as representing osteomyelitis at a femoral catheter site, but a bone

biopsy revealed fibrous dysplasia. The sacral café au lait spot noted at birth had darkened and enlarged, and new spots had appeared. The diagnosis of MCAS with primary hyperthyroidism was made and treatment with propylthiouracil was started. Resolution of tachycardia and normalization of thyroid function occurred and levothyroxine therapy was begun.

When the patient was 4 months of age, serum cortisol concentration was 940 nmol/L (34 μg/dl) and was not suppressed by a high dose of dexamethasone. Adrenocorticotropic hormone, urinary free cortisol, and results of adrenal ultrasonography and computed tomography were normal. By 10 months of age the patient had a cushingoid appearance, with diffuse osteopenia and growth failure. The urinary free cortisol level was 2700 μg/gm creatinine (normal, 20 to 100) and was not suppressed by dexamethasone. Serum adrenocorticotropic hormone was undetectable. The patient was initially treated with aminoglutethimide and metyrapone, and underwent bilateral adrenalectomy at age 2 years.

Although the patient's early tachycardia and hypertension resolved with control of his thyroid and adrenal disease, a systolic ejection murmur and mild cardiomegaly persisted throughout life. The patient also had a chronic mild hypochromic microcytic anemia and α-thalassemia trait.

Because of direct hyperbilirubinemia (total, 277 μmol/L [16.2 mg/dl]; conjugated, 178 μmol/L [10.4 mg/dl]) and unexplained neonatal elevation of serum liver enzymes (aspartate aminotransferase, 447 U/L; alanine aminotransferase, 873 U/L; γ-glutamyltransferase, 3800 U/L; alkaline phosphatase, 1620 U/L; 5′-nucleotidase, 141 U/L), the patient underwent a liver biopsy at 4 weeks that showed canalicular and hepatocellular cholestasis. A trial of phenobarbital therapy was initiated in an effort to stimulate bile flow. Neonatal disophenin scan showed normal uptake with poor excretion, suggesting cholestasis. Another scan at 6 months of age showed only slightly delayed excretion. Hyperbilirubinemia resolved during the neonatal period, but liver biopsies at ages 21 and 24 months revealed chronic cholestasis and progressive fibrosis, and results of liver function tests remained abnormal (e.g., AST 197 U/L, ALT 241 U/L, and GGT 1690 U/L at 24 months). A single gallstone detected by ultrasonography was removed during adrenalectomy. The common bile duct appeared normal at that time.

The patient never had signs of sexual precocity, and skeletal maturation was normal for age. The patient had progressive polyostotic fibrous dysplasia, with shepherd's crook deformities, pathologic long bone fractures, and basal skull sclerosis. He was never able to bear weight or walk. There was no evidence of hypophosphatemic rickets. Computed tomography scan of the brain at 5 months of age showed mild, diffuse atrophy. Developmental evaluation at 2 years of age showed function at a 1-year level. The patient had profound growth failure until age 2½ years, at which time growth velocity accelerated. At age 3 years 7 months, he had reached the 5th percentile for height and weight but not for head circumference. A random determination of growth hormone level at that time was 17 μg/L, and additional studies to rule out a pituitary adenoma were planned.

During what appeared to be a minor viral illness at age 3 years 10 months, the patient had an unexplained cardiac arrest and died. An autopsy did not reveal the cause of death. The pituitary gland showed multifocal hyperplasia, with the possibility of early, focal adenoma formation; immunostaining of the nodular areas showed only growth hormone and prolactin. The thyroid showed multinodular colloid goiter. The liver was abnormally large and had extensive focal nodular hyperplasia and bridging fibrosis, chronic cholestasis, and bile duct paucity. The heart was grossly normal, but microscopic section of the left ventricle showed hypertrophied myocytes with bizarre nuclei, predominantly limited to the epicardial third of the ventricle. Other findings at autopsy included bilateral pulmonary hemorrhages and congestion, a small accessory spleen, and double right ureter.

An Arg^{201}-to-His mutation was detected in all endocrine tissues: hyperplastic pituitary and adrenal glands, thyroid goiter, and testes (Fig. 1). A high proportion of mutant allele was present in abnormal specimens of liver and left ventricle. The mutation was barely detectable in a section of normal-appearing thymus.

Patient 2. Patient 2, a white female infant, was the 1958 gm product of an uncomplicated 38-week gestation. Apgar scores were normal. Birth weight, length, and head circumference were at the 50th percentile for a 33-week gestation. The patient was admitted to the hospital at age 10 days because of dehydration and watery diarrhea and was found to have hyponatremia, hypokalemia, hypophosphatemia, and profound metabolic acidosis. Seizures were treated with phenobarbital. Results of screening tests for inherited metabolic diseases were negative. Fanconi syndrome was diagnosed, and treatment with phosphate, sodium citrate, and potassium citrate was begun. An echocardiogram obtained because of a heart murmur revealed left ventricular hypertrophy.

At 2 months of age the patient was readmitted for evaluation of severe growth failure, facial hirsutism, and multiple café au lait spots. Skeletal survey revealed severe osteopenia but no fibrous dysplasia. Serum cortisol (2050 nmol/L; 74.4 μg/dl) and 24-hour urinary free cortisol (290 nmol; 104 μg) levels were markedly elevated and remained so during a 2-day high-dose dexamethasone suppression test, consistent with autonomous adrenal function. Bilateral

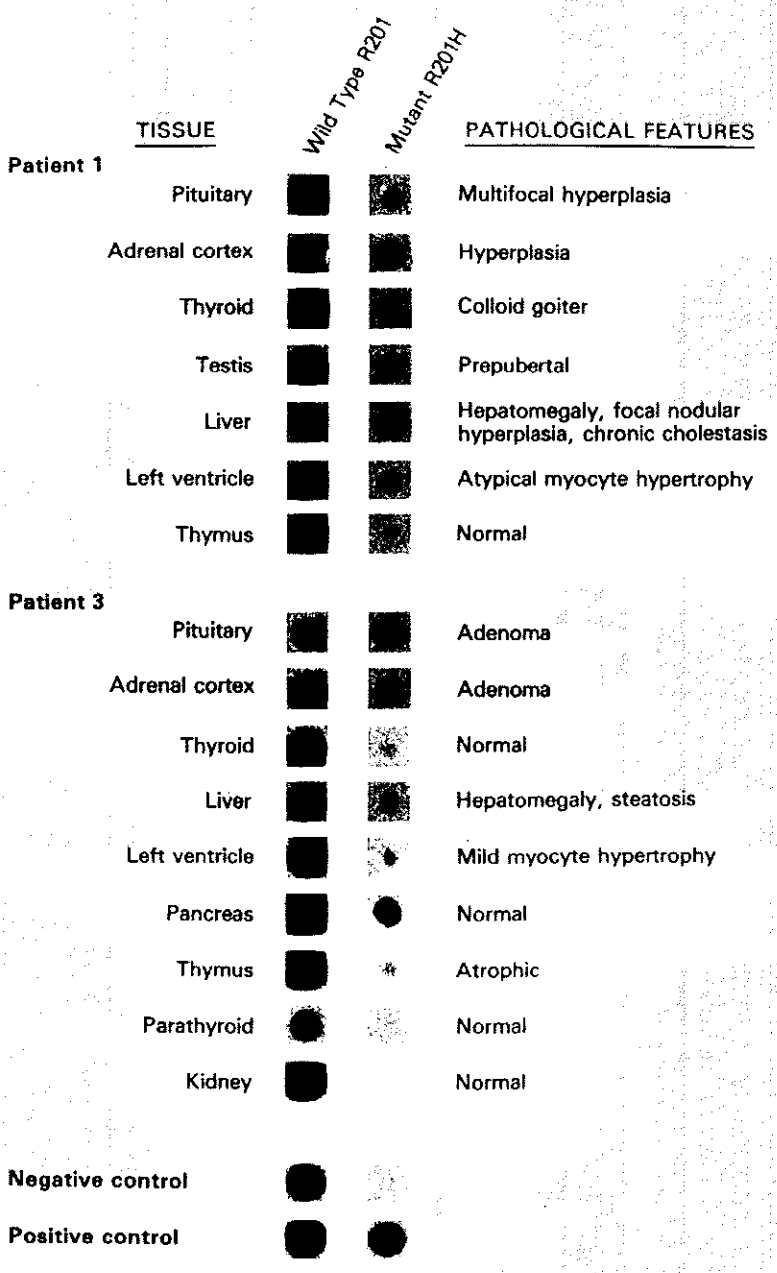

Fig. 1. Analysis of DNA samples from patients 1 and 3. Polymerase chain reaction–amplified genomic fragments encompassing exon 8 from paraffin-embedded tissues from patients 1 and 3 and from control tissues were hybridized with wild-type R201 and mutant R201H oligonucleotide probes. The pathologic changes in each tissue are summarized on *right*. There was no hybridization of DNA with the mutant R201C oligonucleotide probe (not shown).

adrenalectomy was performed at age 3 months, revealing macronodular adrenocortical hyperplasia.

During the same hospitalization, the thyroxine concentration was 238 nmol/L (18.5 μg/dl), triiodothyronine 6.8 nmol/L (440 ng/dl), and thyrotropin <1 mU/L. Thyrotropin remained undetectable after intravenous infusion of thyrotropin releasing hormone, indicating autonomous thyroid function. Initial therapy consisted of propylthiouracil and propranolol. The patient was treated with iodine 131 at age 6 months, and subsequently with potassium iodide and methimazole. Subtle signs of hypothyroidism appeared at age 17 months, but she did not tolerate treatment with a low dose of levothyroxine.

Elevated activity of serum liver enzymes was noted dur-

ing the first month of life and remained elevated (AST 62 to 193 U/L, ALT 192 to 436 U/L, GGT 771 to 2590 U/L, alkaline phosphatase 1308 U/L, 88% heat stable). At age 3 months there was also a transient direct hyperbilirubinemia (total, 65 μmol/L [3.8 mg/dl]; conjugated, 46 μmol/L [2.7 mg/dl]), and liver biopsy revealed marked canalicular cholestasis and extramedullary hematopoiesis.

During the first year of life the patient had several episodes of vaginal spotting. At 16 months of age, she began to develop breast tissue. The serum estradiol concentration was elevated, and gonadotropin responses were suppressed after administration of gonadotropin releasing hormone. Pelvic ultrasonography revealed uterine enlargement and a left ovarian cyst. The patient was treated with testolactone.

At age 6 months a skeletal survey revealed severe osteopenia and rickets; the serum phosphate concentration was 0.8 mmol/L (2.6 mg/dl), serum calcium concentration was 2.50 mmol/L (10.0 mg/dl), plasma parathyroid hormone concentration was normal, and severe renal phosphate wasting was documented (tubular reabsorption of phosphate = 15%; maximal tubular reabsorption of inorganic phosphate, normalized according to glomerular filtration rate = 0.2 mmol/L). The patient was treated with orally administered phosphate and 1,25-dihydroxyvitamin D, but osteopenia persisted. There was a progression of severe polyostotic fibrous dysplasia, and the patient had a fracture of the right leg. Phosphate and vitamin D therapy was discontinued at age 18 months, serum calcium and phosphate levels remained normal for the 3 months, and maximal tubular reabsorption of inorganic phosphate, normalized according to glomerular filtration rate, was found to have improved to 1.4 mmol/L. The serum phosphate concentration subsequently fell to 1.1 mmol/L (3.5 mg/dl) and therapy was restarted.

Other medical problems included growth retardation, feeding intolerance, gastroesophogeal reflux, and suspected allergy to cow milk. An episode of pneumocystis pneumonia at age 4 months that required mechanical ventilation was attributed to immunosuppression from hypercortisolism. At age 2 years the patient stopped gaining weight, and hypertension, tachycardia, and excessive sweating developed. Tests for hyperthyroidism and pheochromocytoma showed negative results.

After ingestion of milk at age 2 years, the patient had persistent vomiting for several hours and turned ashen. Supplemental steroids were given, and the child was brought to the hospital emergency department, where she died. The family declined an autopsy.

The results from the only two specimens that were available for analysis, a surgical block of hyperplastic adrenal gland and frozen skin fibroblasts derived from a nonpigmented area, are shown in Fig. 2. An Arg^{201}-to-Cys mutation was found in both specimens. The ratio of normal to mutant alleles in the skin sample indicated that the specimen contained both normal and abnormal cells.

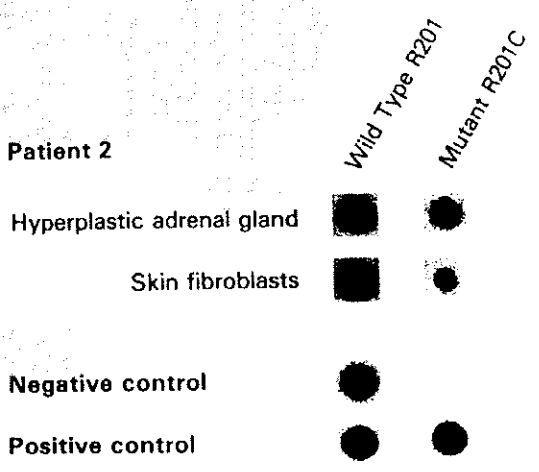

Fig. 2. Analysis of DNA samples from patient 2. Polymerase chain reaction–amplified genomic fragments encompassing exon 8 from samples from patient 2 and from control tissues were hybridized with wild-type R201 and mutant R201C oligonucleotide probes. There was no hybridization of DNA with the mutant R201H oligonucleotide probe (not shown).

Patient 3. Patient 3, a male adolescent with polyostotic fibrous dysplasia, café au lait spots, and a pituitary adenoma, was initially described by Joishy and Morrow.[20] The patient had been hospitalized for 9 days at age 16 years with an episode of acute pancreatitis. Serum amylase and lipase activities were elevated, and serum liver enzyme values were normal. The pancreatitis was attributed to mild hypertriglyceridemia on the basis of serum lipoprotein electrophoresis that showed a prominent prebeta-lipoprotein band (23%) and a serum triglyceride level of 1.4 mmol/L (125 mg/dl). In retrospect, this explanation seems unlikely because the incidence of pancreatitis associated with triglyceride levels less than 1000 mg/dl is rare.[21]

One year later, immediately after surgery to remove a pituitary adenoma, a wound infection developed and the patient died of sepsis.[20] Immunostaining of the pituitary adenoma for hormones showed only growth hormone and prolactin. The autopsy revealed signs of terminal septicemia, adrenal nodular hyperplasia, and hepatomegaly with marked steatosis. The heart was grossly normal, but microscopic section of the left ventricle showed mild myocyte hypertrophy. The nuclear enlargement was less impressive than that seen in patient 1, and was focally distributed throughout the ventricle. Although the autopsy report described fibrotic changes in the pancreas, the one block that was available for analysis appeared normal except for some concretions in the duct.

Analysis of DNA prepared from autopsy material (Fig.

1) revealed the presence of the Arg^{201}-to-His mutation in the adenomatous pituitary and adrenal glands. Mutant alleles were also present in the large, fatty liver but were less abundant in thyroid and heart tissue. The proportion of mutant allele in tissue from the pancreas was high. Mutant allele was also detected in the atrophic thymus but not in the normal parathyroid and kidney. The testes were not available for analysis.

DISCUSSION

In terms of the extent of disease, its early onset, and the fatal outcome, patients 1 and 2 represent two of the most severely affected patients with MCAS reported. As with several other published cases,[4, 22] there was evidence that the disease process began in utero, but none of the "classic" features of MCAS was immediately prominent. Patients 1 and 2 both had complex symptoms that eluded early diagnosis, but autonomous endocrine hyperfunction was soon recognized in both. The diagnosis of MCAS should be entertained in any infant with hypercortisolism or hyperthyroidism.

The endocrine abnormalities in all three of our patients are typical of those described in MCAS[3, 4] and were associated with the presence of the Arg^{201} mutation. Mutation was not detected in most normal-appearing tissues. The fact that males with MCAS do not have sexual precocity as often as females has been described previously,[4] and sexual precocity was not observed in either of our two male patients, although a high proportion of cells in the testis of patient 1 harbored the mutation. All three patients had significant nonendocrine abnormalities and died prematurely.

It is often forgotten that two of the patients described by McCune and Bruch[1] and by Albright[2] (see also references 14 and 23) first came to medical attention because of neonatal, nonendocrine disease. McCune and Bruch's patient had severe, transient jaundice of such intensity that the diagnosis of congenital biliary atresia was entertained.[1] Albright's patient had diarrhea, failure to thrive, microcephaly, and developmental delay.[23] Both patients were soon recognized to have the triad of polyostotic fibrous dysplasia, café au lait spots, and sexual precocity that came to define MCAS, and both children died before reaching adolescence. In an insightful early review of fibrous dysplasia, Lichtenstein and Jaffe[13] delineated a subset of patients with early, extensive bone disease and short life expectancy. They predicted that extended forms of the syndrome might represent a "museum of developmental abnormalities."[13]

In the past 50 years, however, only a few reports have focused on the significance of nonendocrine abnormalities associated with MCAS.[4, 14-16] The syndrome has generally been considered nonfatal, and two long-term follow-up studies did not suggest that patients were at increased risk for nonendocrine disease or premature death.[17-19] The fact that some of the earliest reported patients died in childhood might be attributed to the lack of sophisticated medical care. It is also possible that, like our patients, they had an intrinsically severe form of the disease associated with increased morbidity and with early death. Because significant liver or heart abnormalities were present in six of the first seven patients whom we studied, we reviewed the literature on MCAS and summarized the occurrence of hepatobiliary abnormalities, cardiovascular disease, and early death in 19 other patients. In the course of our work, we became aware of three other cases with one or more of these features; all 28 cases are recorded in the Table. The survey emphasizes the existence of a subset of severely affected patients with MCAS who may be at risk for early death. The Arg^{201} mutation has been present in all seven liver and all four heart specimens that have been analyzed.

Severe neonatal jaundice, elevated serum liver enzyme values, and liver abnormalities have been described, alone or in combination, in at least 16 patients with MCAS. Although liver biopsy specimens from the first child in our previous study were unrevealing,[10] the results of fast atom bombardment–mass spectrometry analysis of urinary bile acids suggested a mild degree of cholestasis, and five of the eight available liver specimens from children with MCAS had cholestatic changes or biliary abnormalities (unpublished results). Extramedullary hematopoiesis, which was noted in the liver of patient 2, has also been seen in four other cases of MCAS (references 24 and 25; unpublished results), but the cause of this phenomenon is unknown.

Hepatobiliary dysfunction appears to be a relatively rare manifestation of MCAS. Of 30 girls with MCAS examined at the National Institutes of Health since 1985, only one has had markedly elevated serum liver transaminase values[10] and two others have had transient, unexplained increases. None of these patients currently has other clinical evidence of liver dysfunction.

Other nonclassic manifestations of MCAS have been observed, and these include gastrointestinal polyps; hyperplasia of the thymus, spleen, and pancreatic islet cells[4, 10, 14-16, 26]; and acute pancreatitis (our patient 3). Unusual clinical manifestations of MCAS, including failure to thrive, metabolic acidosis, abnormalities in serum electrolyte, glucose, or insulin levels, developmental delay, and microcephaly,[4, 23, 24, 27-32] may be related to severe endocrine dysfunction or could be a direct consequence of an Arg^{201} mutation in nonendocrine tissues. The cause of the neuroanatomic and behavioral abnormalities in some patients with MCAS[4, 14, 28, 33] deserves further investigation.

Cyclic AMP stimulates the growth and function of endocrine tissues classically involved in MCAS, including the gonads, thyroid gland, adrenal cortex, and pituitary soma-

Table. Hepatobiliary disease, cardiac disease, and early death in MCAS

Patient No.	Sex	Hepatobiliary disease	Cardiac disease	Outcome	Reference or PC
1	F	+	+	Died, age 12 yr	1,57
2	F	0	+	Died, age 10 yr	2,14,23
3	M	0	+	Postoperative death, age 11 yr, after thyroidectomy	Barnwell and Musser (cited in references 14, 58
4	M	+	0	Unknown	29
5	F	+	0	Unknown	29
6	F	+	0	Unknown	59
7	M	0	0	Died, age 5 yr	28
8	M	0	+	Died, age 32 yr	60
9	F	0	0	Died, age 15 yr	17, 18
10	F	0	+	Unknown	37
11	F	0	+	Alive, age 66 yr	37; PC: Dr. George J. Ellis
12	M	0	+	Died, age 53 yr	31
13	M	0	+	Unknown	38
14	F	0	0	Postoperative death, age 19 yr, from "hemorrhagic complications"	26
15	F	+	0	Alive, age 52 yr	35
16	F	+*	0	Unknown	25
17	F	+	+	Died, age 4 mo	22
18	F	+	0	Alive, age 4 yr	10
19	M	+	+	Sudden death, age 17 yr	10,15
20	M	+	+	Sudden postoperative death, age 5 yr	3,10
21	M	0	+	Thyroid storm; persistent, unexplained tachycardia	61,62; PC: Dr. Robert Richman
22	M	+	+	Sudden death, age 3 yr	Patient 1, this report
23	F	+	+	Died, age 2 yr	Patient 2, this report
24	M	+	+	Postoperative death, age 17 yr, from sepsis	Patient 3, this report
25	F	+	0	Alive, age 43 yr, after liver transplant for cirrhosis and liver failure attributed to hepatitis C	63; PC: Dr. Michael Levine
26	F	0	+	Alive, age 14 yr, unexplained tachycardia and hypertension	64 (patient 3); PC: Dr. Merrily Poth
27	M	+	0	Alive, age 2 yr	65; PC: Dr. Bruce Boston
28	F	+	+	Died, age 4 mo	PC: Dr. Georgeanna Klingensmith

PC, Personal communication.
*Extramedullary hematopoiesis.

totrophs. The Arg^{201} mutations have been documented in abnormal specimens from all these tissues,[10] as well as in pigmented skin macules[11] and dysplastic bone[34] from patients with MCAS. Although it has been proposed that a substance elaborated by dysplastic bone is responsible for the diminished phosphate reabsorption and hypophosphatemic rickets observed in some patients with MCAS (e.g., patient 2), it seems more likely that constitutively activated mutant $G_S\alpha$ subunits in proximal renal tubules are responsible.[3, 4, 35] Unfortunately, samples of kidney from such patients have not been available to test this hypothesis directly.

The presence of cells expressing mutant G_S proteins may also play a role in the significant nonclassic manifestations of MCAS. Although five patients who died early had previously undergone adrenalectomy for Cushing syndrome, and one other who died was being treated for this condition medically,[22] there is no evidence that these children had inadequate glucocorticoid replacement. Hypocortisolism, hypercortisolism, hyperthyroidism, growth hormone excess, or arteriovenous shunting through dysplastic bone may contribute to the cardiovascular complications in MCAS,[36-38] but it is also plausible that the presence of cardiac cells harboring the G_S mutation in some patients directly in-

creases their risk of premature or sudden death. The cardiotoxic and dysrhythmic effects of excess cyclic AMP are well known (reviewed by Packer et al.[39]). The presence of cardiac cells with the G_S mutation may help explain the unusual myocyte hypertrophy in patient 1 and the cardiomegaly and persistent tachycardia described in several other patients with MCAS (Table).

The mechanism by which activated G_S might cause hepatobiliary dysfunction remains unclear. Cyclic AMP is involved in the control of hepatocyte gluconeogenesis,[40] collagen metabolism,[41] proliferation,[42] and plasma membrane potential,[43] and in the formation of bile by both hepatocytes[44-47] and bile duct epithelial cells.[48, 49] There is evidence that artificially sustained elevation of cyclic AMP per se does not cause leakage of hepatocellular enzymes.[44, 45] It is conceivable that the presence of inappropriately activated G_S causes abnormal embryologic development of the biliary system, and that this leads to neonatal cholestasis and other, secondary pathologic effects. Although cyclic AMP has a role in bicarbonate secretion by pancreatic duct cells,[50] the reason that chronic overstimulation of this pathway might lead to an episode of acute pancreatitis, such as the one in patient 3, is not clear. The role of $G_S\alpha$ mutation in other unusual MCAS abnormalities, including extramedullary hematopoiesis and neuroanatomic manifestations, remains obscure.

It may be an oversimplification to consider only the potential effects of excess cyclic AMP when evaluating the pathophysiologic consequences of the activating $G_S\alpha$ mutation. The G_S protein modulates the activity of ion channels[12] and a membrane calcium pump,[51] and there may be roles for G_S in cellular differentiation[52] and intracellular membrane trafficking[53] that are not mediated by cyclic AMP. In addition to impairing guanosine triphosphatase activity, mutations at Arg^{201} can decrease membrane attachment of $G_S\alpha$, increase its degradation,[54] and affect its interaction with $\beta\gamma$ subunits.[55]

We conclude that the widespread, heterogeneous distribution of abnormal cells containing the Arg^{201} mutation in both endocrine and nonendocrine tissues of patients with MCAS is consistent with the occurrence of a somatic mutation of the $G_S\alpha$ gene during embryogenesis. Severe disease may be associated with an earlier mutational event that leads to a larger number or a more widespread distribution of mutant cells. Mosaicism for single gene defects may represent a generally important mechanism of disease.[56] Manifestations of severe MCAS are more extensive than is generally appreciated and may include hepatobiliary, cardiac, and pancreatic disease, neurodevelopmental abnormalities, and other nonendocrine dysfunction, as well as sudden or premature death. Further attention should be focused on the pathophysiologic consequences of constitutive activation of G_S in nonendocrine MCAS tissues, because this may serve as a model for understanding the role of G_S signaling pathways in other human diseases.

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